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REVIEW ARTICLE

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Ethylene glycol: an estimate of tolerable levels of exposure based on a review of animal and human data

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Abstract Upon ingestion ethylene glycol (EG, monoethylene glycol) is rapidly absorbed from the gastrointestinal tract, and depending on the severity of exposure signs of toxicity may progress through three stages. Neurological effects characterize the first step consisting of central nervous depression (intoxication, lethargy, seizures, and coma). The second stage, usually 12–24 h after ingestion, is characterized by metabolic acidosis due to the accumulation of acidic metabolites of EG, primarily glycolic acid (GA), contributing to the ensuing osmolal and anion gaps. Stage 3, generally 24–72 h after ingestion, is determined mainly by oxalic acid excretion, nephropathy, and eventual renal failure. Because the toxicity of EG is mediated principally through its metabolites, adequate analytical methods are essential to provide the information necessary for diagnosis and therapeutic management. The severe metabolic acidosis and multiple organ failure caused by ingestion of high doses of EG is a medical emergency that usually requires immediate measures to support respiration, correct the electrolyte imbalance, and initiate hemodialysis. Since metabolic acidosis is not specific to EG, whenever EG intoxication is suspected, every effort should be made to determine EG as well as its major metabolite GA in plasma to confirm the diagnosis and to institute special treatment without delay. A number of specific and sensitive analytical methods (GC, GC-MS, or HPLC) are available for this purpose. Due to the rapid metabolism of EG, the plasma concentration of GA may be higher than that of EG already upon admission. As toxicity is largely a consequence of metabolism of EG to GA and oxalic acid, the simultaneous quantification of EG and GA is important. Formation of calcium oxalate monohydrate in the urine may be a useful indicator of developing oxalate nephrosis although urine crystals can result without renal injury. The pathways involved in the metabolism of EG are qualitatively similar in humans and laboratory animals, although quantitative differences have been reported. Comparison between species is difficult, however, because the information on humans is derived mainly from acute poisoning cases whereas the effects of repeated exposures have been investigated in animal experiments. Based on published data the minimum human lethal dose of EG has been estimated at approx. 100 ml for a 70-kg adult or 1.6 g/kg body weight (calculation of dose in ml/kg to mg/kg based in EG density = 1.11 g/l). However, human data from case reports are generally insufficient for the determination of a clear dose-response relationship and quantification of threshold doses for systemic toxicity, in particular renal effects, is limited. As toxicity is largely a consequence of metabolism of EG to GA, it is important to note that no signs of renal injury have developed at initial plasma glycolate concentrations of up to 10.1 mM (76.7 mg/dl). Plasma EG levels of 3.2 mM (20 mg/dl) are considered the threshold of toxicity for systemic exposure, if therapeutic strategy is based on the EG concentration alone.

Keywords Ethylene glycol · Glycolic acid · Analysis · Toxicokinetics · Nephrotoxicity

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Introduction

Although ethylene glycol (EG) poisoning is uncommon in medical practice, it is an important condition to recognize because early diagnosis and appropriate therapeutic intervention can prevent severe morbidity and mortality. Individuals may consume EG accidentally or intentionally, usually in the form of antifreeze products

or as an ethanol substitute. The conditions of exposure are often difficult to ascertain, as case reports of EG poisoning are deficient in many areas. Only a minority of cases include an estimate of the oral intake and the time course of development of toxic symptoms. The presence of metabolites, essential for causal therapy, is often insufficiently described. This applies particularly to glycolic acid (GA), the only toxic metabolite that accumulates to any extent in human plasma. The level of GA directly relates to increased anion gap, severity of symptoms and mortality. Moreover, the plasma concentrations of EG and GA are dependent on time from poisoning to admission and analysis, as well as on simultaneous ingestion of ethanol, antidote treatment (ethanol, fomepizole) or institution of hemodialysis.

Misidentification of EG has occurred in several clinical cases, for example, with 2,3-butanediol (Jones et al. 1991) and propionic acid arising from inborn methylmalonic acidemia (Shoemaker et al. 1992). Similarly, interference between GA and plasma lactate has been reported in an enzyme-based assay (Porter et al. 2000). In order to appreciate the importance of selective assays for the determination of EG and GA for both diagnosis and therapeutic intervention based on toxicokinetic considerations, the analytical methods are reviewed in the first instance. The data obtained in cases of EG poisoning are then related to the occurrence of systemic toxicity, especially nephrotoxicity, and its possible dose-response relationship.

Analytical methods

EG is commonly analyzed after derivatization by gas chromatography (GC) using either a flame ionization detector or mass spectrometry (MS) for quantification. The metabolites of EG, glycolic acid in particular, are determined in body fluids by high-performance liquid chromatography (HPLC), GC, or GC-MS. Selected methods for EG and glycolic acid are summarized in Tables 1 and 2, respectively. The presently preferred method for determination of EG by GC is based on phenylboronation (Hewlett et al. 1986; Porter and Auansakul 1982). When the analysis is performed with a commonly used capillary column, it appears to be free from known interferences (Porter et al. 1994). Other methods such as those based on direct injection may be subject to carryover (Aarstad et al. 1993) or significant interferences from physiological substances, for example, propionic acid (present in methylmalonic acidema; Shoemaker et al. 1992) and 2,3-butanediol (present in serum from alcoholic patients; Jones et al. 1991).

For the rapid determination of EG an enzymatic method using glycerol dehydrogenase has been proposed (Hansson and Masson 1989). However, interferences with the enzymatic assay are to be expected from glycerol or propylene glycol (used as solvents for parenteral drugs) or β-hydroxybutyrate (present in serum from patients with diabetic ketoacidosis; Allen and Hamlin

Table 1 Analytical methods for determining ethylene glycol (EG) in human samples

Sample	Method of preparation	Analytical method	Limit of detection (quantitation)	Recovery	Reference
Serum	Derivatization with phenylboronic acid after addition of internal standard: 1,3-propanediol	GC	0.2 mM (12.4 μg/ml)	Not recorded	Hewlett et al. 1986
Serum	Protein precipitated by acetonitrile, water in supernatant eliminated by a mixture of 2,2-dimethoxypropane:*N*,*N*-dimethylformamide:acetic acid (78:20:2 v/v). Volume of residue reduced under nitrogen at 60°C; sample derivatized with N, *O*-bis(trimethylsilyl/trifluoroacetamide solution containing trimethylchlorosilane (10 ml/l). Internal standard: 3-bromo-1-propanol	GC	0.08–0.16 mM (5–10 μg/ml)	Absolute: 91.0–91.2%; relative to calibrator: 98.9–103.8%	Yao and Porter 1996
Serum	Protein precipitated by acetic acid/acetonitrile (1:10 v/v); water in supernatant eliminated by a mixture of 2,2-dimethoxypropane:*N*, *N*-dimethylformamide:acetic acid (78:20:2 v/v). Volume of residue reduced at 80°C; sample derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide. Calibrators: EG, glycolic acid	GC-MS	0.16 mM (10 μg/ml)	91.1%	Porter et al. 1999

Table 2 Analytical methods for determining glycolic acid (GA) in human samples

Sample	Method of preparation	Analytical method	Limit of detection	Recovery	Reference
Serum	Glycolic acid extracted from salted, acidified serum using ethyl methyl ketone followed by removal of organic phase and evaporation to dryness. Residue dissolved in ethyl acetate and derivatized with *O-p*-nitro-benzyl-*N*, N-diisopropylisourea (PNDBI)	HPLC/MS	0.05 mM (3.8 μg/ml)	Not recorded	Hewlett et al. 1983, 1986
Serum	Protein precipitated by acetonitrile, water in supernatant eliminated by a mixture of 2, 2-dimethoxypropane:*N*,*N*-dimethylformamide:acetic acid (78:20:2 v/v). Volume of residue reduced under nitrogen at 60°C; sample derivatized with *N*, *O*-bis(trimethylsilyl/trifluoroacetamide solution containing trimethylchlorosilane (10 ml/l). Internal standard: 3-bromo-1-propanol	GC	0.06–0.13 mM (5–10 μg/ml)	Absolute: 77.3–81.7%; relative to calibrator: 95.1–104.9%	Yao and Porter 1996
Serum	Protein precipitated by acetic acid/acetonitrile (1:10 v/v); water in supernatant eliminated by a mixture of 2,2-dimethoxypropane:*N*,*N*-dimethylformamide:acetic acid (78:20:2 v/v). Volume of residue reduced at 80°C; sample derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide. Calibrators: EG, GA	GC-MS	0.13 mM (10 μg/ml)	94.2%	Porter et al. 1999
Plasma	Following protein precipitation by trichloroacetic acid, glycolic acid in supernatant is derivatized to methyl glycolate and then analyzed using methyl propionate as internal standard	GC	0.33 mM (25 μg/ml)	99–112%	Fraser and MacNeil 1993; Moreau et al. 1998

1993; Blandford and Desjardins 1994; Malandain and Cano 1995; Nilsson and Jones 1992).

Determination of GA by HPLC-MC (Hewlett et al. 1983; Petrarulo et al. 1991) involves complex and rather laborious methodology. Other methods based on isotachophoresis (Øvrebø et al. 1987) or on ion chromatography (Hagen et al. 1993) are not available in many laboratories. An enzymatic method using glycolate oxidase (Kasidas and Rose 1979) is not specific due to lactate interference.

More recently a GC procedure with flame ionization detection for the simultaneous determination of EG and GA in serum has been proposed that appears suitable for clinical use (Yao and Porter 1996). The method, based on trimethylsilylation of EG, glycolic acid or 3-bromo-1-propanol (internal standard), has a high assay precision and is virtually free of interferences. Of more than 60 potentially interfering compounds for which retention times of their trimethylsilyl derivatives have been determined only 3-dimethyl-amino-1-propanol partly overlapped with EG and 1,2-butanediol coeluted with GA. Neither of these compounds is naturally occurring or present in medications. The same laboratory recently reported an adaptation of this procedure using the more specific mass spectral detection instead of flame ionization detection (Porter et al. 1999). In this procedure EG and GA are specifically analyzed as the *t*-butyldimethylsilyl derivatives. These derivatives are more stable than the trimethylsilyl derivatives, have better GC resolution and they have more characteristic mass spectra.

In other GC methods GA is derivatized to methylglycolate and analyzed using methyl propionate as internal standard (Fraser and MacNeil 1993; Moreau et al. 1998). The limit of quantitation of GA by GC analysis was 0.33 mM with a mean confidence value of 7% and recovery from plasma that was practically complete (Moreau et al. 1998).

The selectivity and sensitivity of GA analyses in human poisoning cases reported in the recent literature has been evaluated (Bartels 2002). In spite of the variety of methods used the determination of GA in plasma/serum was fairly accurate. The highly specific GC-MS assays generally provided confirmation of the analytical results with another method. The detection limit for GA by the various assays ranged from 0.05–7 mM, with most between 0.13–2.8 mM (1.0–21.3 mg/dl). This level of sensitivity is generally well below the levels of GA reported in such cases (see also Table 4).

It is noted that only a few clinical toxicology laboratories routinely offer such measurements. In the acute setting of managing the intoxicated patient the use of serum EG (determined enzymatically or with an automated analyzer), anion, and osmolal gaps coupled with arterial blood gas measurements and urinalysis are expected to provide the greatest diagnostic yield within an acceptably short time period (Wolf and Shaw 1998). Recent national survey in the United States found that only 25% of 95 teaching hospitals perform EG deter-

minations with a median turnaround time of 1.5 h, whereas if the test is sent out, the time is 42 h (Kearney et al. 1997).

Toxicokinetics and metabolism of ethylene glycol

To understand the relevance of glycolic acid levels and their bearing on the course of EG intoxication it is important to discuss the toxicokinetics and metabolism of EG in regard to prime parameters indicative of metabolic and organ damage. EG is rapidly absorbed from the gastrointestinal tract and metabolized. Its half-life in plasma is estimated at 3–7 h in laboratory animals (Marshall 1982; Winek et al. 1978) and appears to be of a similar order in humans. Upon self-administration of 8, 10, and 12 ml EG by a male volunteer, serum $t_{1/2}$ was 4.5 h with 2.3% of the dose eliminated in the urine as oxalic acid (Reif 1950). Data on toxicokinetic parameters for EG compiled from the more recent literature are consistent with $t_{1/2}$ of 3–8.6 h, V_d 0.5–0.8 l/kg, and mean renal clearance between 0.75 ml/min (renal failure) and 27.5 ml/min (normal renal function; Eder et al. 1998). In general the ratio of urinary to serum EG concentration is high, and renal clearance values of 17–70 ml/min have been reported (Cheng et al. 1987; Harry et al. 1994).

EG is first metabolized to glycolaldehyde by hepatic alcohol dehydrogenase, then to GA via aldehyde dehydrogenase. GA is subsequently oxidized to glyoxylic acid and then oxalic acid (Fig. 1). Further suggested pathways in humans involve the conversion of glycolaldehyde to glyoxal, glyoxylate to glycine (and its conjugate hippurate in the presence of benzoic acid) and, theoretically, to formic acid (Gabow et al. 1986). The metabolites may independently cause toxicity in laboratory animals (Bove 1966). However, GA is the only toxic metabolite that accumulates to any extent in the plasma of monkeys (Clay and Murphy 1977) and rats (Chou and Richardson 1978) as well as in humans poisoned with EG (Jacobsen et al. 1984). Thus both experimental and human studies indicate that GA metabolism to glyoxylate is rate-limiting in the metabolic pathway of EG (Moreau et al. 1998). In contrast, both glycolaldehyde and glyoxylate have very short half-lives (Chou and Richardson 1978; McChesney et al. 1972), the former being oxidized to GA, while glyoxylic acid is metabolized via diverse pathways to oxalic acid, hippuric acid (via glycine) or possibly other metabolites.

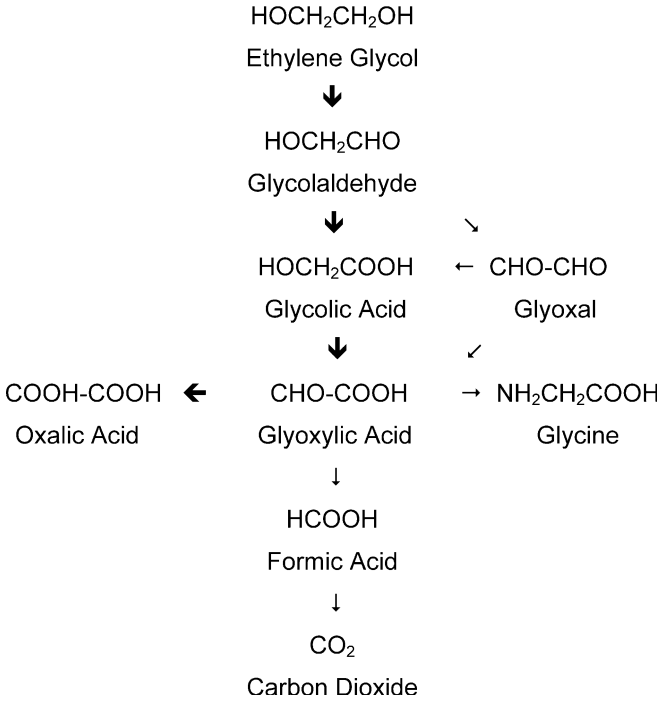

Fig. 1 Metabolism of ethylene glycol. *Bold arrows indicate the major pathway*

Because of its rapid metabolism EG may be low or undetectable in plasma/serum while GA attains high levels (Fraser and MacNeil 1993; Hewlett et al. 1986). The elimination half-life of EG is increased more than tenfold in the presence of ethanol because both compounds compete for the active site of alcohol dehydrogenase (Eder et al. 1998; Jacobsen et al. 1988). This enzyme has a much greater affinity for ethanol than for EG and concentrations of ethanol as low as 11 mM (50 mg/dl) saturate the enzyme (Peterson et al. 1981). Because of the high capacity of alcohol dehydrogenase to oxidize EG in the absence of a competitive inhibitor the measurement of GA in addition to that of EG is important for diagnosis and therapy of intoxication. In fact the serum concentration of GA has been shown to be correlated more closely with the clinical symptoms than the concentration of EG (Hewlett et al. 1986).

The elimination kinetics of EG in human subjects was analyzed in the context of establishing the efficacy of alcohol dehydrogenase inhibition (Sivilotti et al. 2000). In 19 patients with EG concentrations of 0.56–34.03 mM, elimination of EG was first order during fomepizole (4-methyl pyrazole) monotherapy ($t_{1/2}$ 19.7 ± 1.3 h) and under these conditions was not affected by the presence of ethanol. The elimination rate was significantly faster ($t_{1/2}$ $< 8.6 \pm 1.1$ h) in the absence of fomepizole and ethanol. EG elimination by the kidneys was proportional to the renal function as estimated by creatinine clearance with a fractional excretion of $25.5 \pm 9.4\%$. All patients with normal serum creatinine concentration at the initiation of fomepizole treatment had rapid rates of renal elimination ($t_{1/2}$ 16.8 ± 0.8 h). Thus renal elimination and/or hemodialysis were the only significant routes of EG elimination as long as fomepizole concentration was maintained above 10 μM (EG/fomepizole ratio $< 100:1$).

GA is converted to glyoxylate (Chou and Richardson 1978; McChesney et al. 1972). This being the rate limiting step and because of its rapid formation from EG, GA may accumulate in the plasma. Moreover, the pathway from glyoxylic acid to formic acid is practically absent in humans and only small amounts of GA appear

as oxalic acid (about 1%) or glycine/benzoic acid in the urine. The calculated volume of distribution of GA is low (0.55 l/kg; Moreau et al. 1998), similar to that of EG. This indicates that the substances remain in the vascular compartment. Both EG and its main metabolite GA are effectively removed by hemodialysis (Jacobsen et al. 1984).

A number of case reports refer to the elimination kinetics of GA following EG poisoning. In ten patients the endogenous elimination rate of glycolate was 1.08 ± 0.67 mmol/l per hour and elimination $t_{1/2}$ was 10.43 ± 7.90 h ($n = 4$). The elimination $t_{1/2}$ during dialysis was reduced to 2.58 ± 0.70 h ($n = 8$), and hemodialysis ($n = 5$) cleared GA at a rate of 10.20 ± 1.38 l/h with flow rates 15–24 l/h (Moreau et al. 1998).

Considerations of interspecies differences are important whenever animal data are extrapolated to man. The elimination half-life for EG in rats at doses from 20-2,000 mg/kg body weight ranges from 1.0–2.5 h (Frantz et al. 1996a, 1996b; Hewlett et al. 1986), while that in acutely intoxicated humans ranges from 3.0 to 8.4 h (Jacobsen et al. 1988; Peterson et al. 1981). In rats the blood levels of GA, the first identified metabolite of EG using GC-MS technique, increased nearly proportionally to the oral dose from 10 to 150 mg/kg EG but increased disproportionately from 500 to 1000 mg/kg EG (Pottenger et al. 2001). EG and GA were dose-dependently eliminated in the urine for doses of 500 mg/kg EG and above. This high-dose effect was probably due to saturation of metabolic conversion of GA to downstream metabolites. Thus the degree of GA accumulation is likely related to GA metabolism to glyoxylate and this rate-limiting step may differ among species.

In fact Booth et al. (2004) have shown using in vitro techniques that metabolism of [1,2-^{14}C]EG by rat liver slices results in higher levels of GA than produced by liver slices from rabbits or humans. With human liver the formation of low level GA was detected in one incubation of slices from one of four donors but only at one extended time point. Glyoxylate was detected with liver slices from all four humans. Human liver tissue was the most effective at metabolizing GA to glyoxylic acid. The ratios of V_{max}/K_m representing the relative clearance of GA from liver tissue, were approximately 14:9:1 for human, rat, and rabbit liver, respectively. These in vitro data suggest that humans are more effective at metabolism of GA to glyoxylic acid than rats or rabbits. Consequently there is less chance for the accumulation of the toxic metabolite GA in humans than in these laboratory animals.

Ethylene glycol poisoning

Accidental or intentional ingestion of sweet tasting EG may occur in adults and in children. In 1996 the American Association of Poison Control Centers recorded 5,548 reports of EG ingestion, 17 of which related to fatalities as a result of suicide or intentional ingestion. About 18% of the reports involved children younger than 6 years (Litovitz et al. 1997). The ingestion of EG may result in inebriating effects followed by severe central nervous system depression, cardiopulmonary failure, acute renal failure, metabolic acidosis, and possibly death (Beasley and Buck 1980). The metabolic acidosis and severe toxicity are the consequence of the metabolism of EG to several organic acids, among which GA is the principal toxic metabolite that accumulates in blood (Gabow et al. 1986; Jacobsen et al. 1984). Rapid and accurate identification of EG or its metabolites is necessary not only for early diagnosis and successful therapy but also to exclude other possible causes of increased anion gap metabolic acidosis (Eder et al. 1998).

Numerous case reports are available on deliberate ingestion of EG but very few report accidental ingestion (zero in 1996, according to the American Association of Poison Control Centers). The information on dose level is generally insufficient for the determination of a clear dose-response relationship. From published data the minimum human lethal dose has been estimated at approximately 100 ml for a 70-kg adult (about 1.4 ml/kg, or 1.6 g/kg; Ellenhorn and Barceloux 1988). An estimate of the human lethal dose of EG based on 42 published fatal cases indicates a range between 30 ml and 1500 ml (personal communication, W. Gulledge, letter to Ethylene Oxide/Ethylene Glycol Panel, American Chemistry Council, Arlington, Va., December 2000). However, survival has been reported after ingestion of up to 1000 ml (Gaultier et al. 1976).

Clinical signs of systemic toxicity following ingestion of EG generally progress in three stages. Central nervous system (CNS) effects are apparent within 0.5–2 h of ingestion and include inebriation (in the absence of detectable ethanol), nausea, vomiting, nystagmus, papillary edema, hyporeflexia, convulsions, and coma. Within 12–24 h after ingestion cardiopulmonary manifestations are observed, including tachycardia, tachypnea, hypertension, pulmonary edema, and congestive heart failure. A generally late event is renal insufficiency occurring 24–72 h after ingestion. Neurological effects suggestive of cranial nerve damage (facial paralysis, impaired vision) may appear 1–2 weeks after a single exposure (Hall and Rumack 2001).

The severity of toxicological effects and the progression from one stage to the next depend on the amount of EG ingested and the timing of medical intervention (Eder et al. 1998). However, the lag period between the stages depends on the time required for the toxic metabolites of EG to accumulate. Therefore there is a poor correlation between serum EG concentration and the clinical course, and death has been reported with virtually undetectable serum EG concentrations (Litovitz et al. 1997). The systemic effects of poisoning depend primarily on the induction of metabolic acidosis and the magnitude of the osmolal gap and anion gap, respectively.

Serum osmolality is determined from the concentration of sodium, urea nitrogen, and glucose according to the formula: [1.86 × sodium (mM) + urea nitrogen (mM) + glucose (mM)]/0.93 = mOsm/kg H_2O (Glasser et al. 1973). In a healthy individual serum osmolality is 270–290 mOsm/kg H_2O. An osmolal gap, i.e., the difference between the actually measured (by freezing point depression) and calculated osmolality, results from the presence of other solutes in the serum that are not considered in this formula. An increase in the osmolal gap, generally considered important when higher than 10 mOsm/kg H_2O, suggests the presence of unmeasured osmotically active substances such as EG, methanol, ethanol or acetone (i.e., in diabetic ketoacidosis; Eder et al. 1998; Hoffman et al. 1993). In the case of EG poisoning the concentration of parent compound accounts for most of the osmolal gap (Jacobsen et al. 1988). Each 16 mM (100 mg/dl) increment in EG concentration contributes about 16 mOsm/kg H_2O, and each 22 mM (100 mg/dl) of ethanol contributes 22 mOsm/kg H_2O to the osmolal gap. As EG is metabolized to acidic intermediates, the osmolal gap is reduced and the anion gap is increased (Jacobsen et al. 1988) because these anions are counterbalanced by sodium and thus are considered in calculating the serum osmolality (Hoffman et al. 1993).

The anion gap is the difference between the sum of the measured cations and the sum of the measured anions: $[(Na^+ + K^+) - (HCO_3^- + Cl^-)]$. In healthy individuals the physiological anion gap is 12–16 mM. The values of this parameter noted in ten patients with EG intoxication ranged from 20–41 mM (Moreau et al. 1998). Organic acids will increase the anion gap and GA has been shown to account for 96% or more of the anion gap in patients poisoned with EG (Gabow et al. 1986; Jacobsen et al. 1984). In fact the initial anion gap is well correlated with initial serum GA ($R^2 = 0.65$, $P = 0.005$; Moreau et al. 1998). Although the presence of metabolic acidosis with an increased anion and osmolal gap is suggestive of EG poisoning, it is not a specific diagnosis. It may be present in methanol poisoning (Jacobsen et al. 1982), alcoholic ketoacidosis (Schelling et al. 1990), diabetic ketoacidosis (Davidson 1992), renal failure (Sklar and Linas 1983), and multiple organ failure (Inaba et al. 1987).

Nephrotoxicity

Mode of action of renal effects

Data from human poisoning cases and repeated-dose toxicity studies in experimental animals indicate that the kidney is a critical organ for the toxicity of EG. Metabolic acidosis and degenerative changes in the kidney (including tubular degeneration with deposition of calcium oxalate) have consistently been observed in a range of species (CICAD 2002). In addition to the adverse effect of metabolic acidosis on renal function, due mainly to GA accumulation, oxalic acid is of particular toxicological significance, although it is only a minor metabolite of EG. Oxalic acid chelates with calcium ions resulting in the deposition of insoluble calcium oxalate crystals in tissues, notably in the kidney. Crystals of calcium oxalate monohydrate and dihydrate precipitate in the proximal convoluted tubules and renal interstitial tissue, whereby the dihydrate is formed only at high concentrations of calcium and oxalate (Burns and Finlayson 1980). Calcium oxalate crystals are considered to be closely associated with the nephropathy developing in delayed cases of EG poisoning (Jacobsen and McMartin 1986; Wiley 1999). Calcium oxalosis may explain the hypocalcemia that is often observed in EG poisoning (Jacobsen et al. 1988).

The histological diagnosis of acute renal failure secondary to EG poisoning depends on the recognition of tubular damage (vacuolar changes predominantly of the proximal tubular epithelium) in association with intratubular birefringent oxalate crystals. In the urine calcium oxalate may be excreted not only as dihydrate (octahedral) crystals but also, characteristically, as monohydrate (needle shaped) crystals. The latter are strongly birefringent and may thus be distinguished from hippuric acid crystals (Eder et al. 1998). Recognition of monohydrate calcium oxalate crystals in the urine facilitates rapid diagnosis of EG ingestion (Terlinski et al. 1981). Thus crystalluria, identified by simple microscopy of the urine, is an important and rather specific diagnostic tool and repeated urinalysis is very useful in the differential diagnosis of an anion gap metabolic acidosis of unknown origin (Jacobsen et al. 1988). In experimental animals, EG-associated renal damage has been observed only at doses greater than those at which there were increases in crystalluria (DePass et al. 1986).

Dose response

Information on the dose-response relationship has been obtained in laboratory animals. In relevant studies quoted in the CICAD (2002) document, the no observed adverse effect level (NOAEL) for renal effects of EG in the most sensitive animal species (male rat) was determined to be a daily oral dose of 200 mg/kg in a pivotal chronic 2-year bioassay (DePass et al. 1986). In a subchronic 13-week study the NOAEL corresponded to an approximate daily intake of 400 mg/kg (Robinson et al. 1990). In an unpublished 16-week subchronic study (Gaunt et al. 1974, quoted in CICAD 2002), the incidence of individual nephrons with degenerative changes was not statistically different from controls up to and including a daily dose of 180 mg/kg. At a daily dose of 715 mg/kg significant treatment-related renal effects occurred, including increased kidney weight, increased water consumption, and urine oxalate excretion. In a recent comparative oral (dietary) toxicity study of 16 weeks duration conducted in male Wistar and F-344

rats EG produced crystal nephropathy at daily dose levels of 1000 and 500 mg/kg. The NOAEL in this study was 150 mg/kg per day for both strains (Mertens 2002).

Pharmacokinetic and metabolism data referred to above indicate that humans may be less sensitive than rodents to the ingestion of EG. However, data on dose-response in humans are virtually absent since kinetic variables have been measured primarily in poisoned individuals. In the absence of sufficient human data dose response may be characterized by animal data. This appears to be feasible on the basis of renal effects that were typically observed in rats. In a 2-year bioassay a daily oral dose of 200 mg/kg has been determined as the NOAEL (DePass et al. 1986). In the CICAD (2002) document a tolerable intake for EG, with regard to the development of histopathological changes in the kidney of male rats, has been derived from Gaunt et al. (1974) based on a benchmark dose$_{05}$ ($BMD_{05,}$ the dose estimated to cause a 5% increase in incidence over the background response rate, calculated according to Howe 1995). A tolerable intake of 0.05 mg/kg body weight per day for EG was based on a BMD of 49 mg/kg per day calculated for the presumed development of renal effects in animals and an uncertainty factor of 1000. However, this tolerable intake was uncertain, owing primarily to lack of information on progression of renal lesions in the most sensitive animal model. However, the BMD_{05} derived from the data of Robinson et al. (1990) range from 316.4 to 501.9 (95% lower confidence limit 85.5) to 214.9 mg/kg per day for individual lesions. Based on these data a human NOAEL of about 200 mg/kg per day for kidney effects caused by oral exposure to EG can be assumed. This estimate is in good agreement with the NOAEL of 150 mg/kg per day determined by Mertens (2002). However, caution should be exerted in directly extrapolating the animal data to man. Little is known on the pathogenesis of the kidney lesions and the toxicokinetic information is insufficient for reliable interspecies comparison.

A cross-sectional study on 33 aviation workers exposed to EG vapor up to 22 mg/m^3 or EG mist up to 190 mg/m^3 during deicing operations found no evidence of renal injury (based on urinary albumin, β-*N*-acetyl-glucosaminidase, β_2-microglobulin and retinal-binding protein), even with the observation in 16 cases of post-shift urine samples that contained EG quantities exceeding 5 mM/M creatinine. Apparently six cases of these higher urinary levels were from individuals who did not wear paper-type masks intended to protect their respiratory passages from exposure to aerosol (mist; Gérin et al. 1997). Assuming continuous exposure and complete absorption (from the respiratory tract and/or skin which is overly conservative), exposure to 22 mg/m^3 vapor and 190 mg/m^3 aerosol could have resulted in daily systemic exposure to 3.14 mg/kg EG from vapor or 27.14 mg/kg EG from aerosol in a 70-kg worker breathing 10 m^3 of air during an 8-h shift.

The putative toxic metabolite in the induction of kidney lesions, oxalic acid, is derived from glyoxylic acid, a further metabolite of GA. There are no comparative kinetic data that can serve as a basis for interspecies scaling to explain possible quantitative differences between humans and rats in the formation and excretion of these metabolites. However, Booth et al. (2004) showed that when using whole liver slice incubations human tissue metabolizes GA more rapidly than rat and rabbit tissue, and that in the human liver slices GA is not detectable following exposures of up to 40 mM EG. The failure to detect GA could be attributed to its effective further metabolism to glyoxylic acid. In principle, glyoxylic acid could also be formed from glyoxal (Fig. 1), but glyoxal was not detected in the human liver slice incubations.

Evidence from a recent follow-up study of 19 consecutive patients with confirmed EG poisoning (Brent et al. 1999) indicated that no signs of renal injury developed in any patient whose initial plasma GA concentration did not exceed 10.1 mM (76.8 mg/dl) or whose serum creatinine concentration was normal. All of the patients in whom renal injury developed had plasma GA concentrations of 12.9 mM or higher (≥98.0 mg/dl). The respective baseline values obtained in these patients prior to fomepizole treatment are given in Table 3. Renal injury was independent of the initial plasma EG concentration, but all patients who had plasma EG concentrations in excess of 8.1 mM (50 mg/dl) underwent hemodialysis. The ratio of urinary to serum EG concentrations is high, and renal EG clear-

Table 3 Baseline characteristics of 19 patients with ethylene glycol poisoning: mean values (range) (Brent et al. 1999)

Characteristic	All patients ($n=19$)	Patients with renal injury ($n=9$)	Patients without renal injury ($n=10$)	P[a]
Sex: M/F	17/2	8/1	9/1	–
Age (years)	41 (19–73)	43 (28–60)	40 (19–73)	–
Time from EG ingestion to treatment with fomepizole (h)	11.4 (6.6–20.8)	15.1 (9.0–20.8)	9.6 (6.6–13.9)	–
Plasma ethylene glycol (mM)	19.8 (3.9–71.9)	15.9 (3.9–34.4)	23.2 (4.2–71.8)	0.34
Plasma glycolic acid (mM)	11.8 (0-34.8)	21.0 (12.9–343.9)	3.7 (0–10.1)	<0.001
Serum creatinine (mM)	132.6 (53.0–291.7)	194.5 (132.6–291.7)	79.6 (53.0–106.1)	<0.001
Arterial pH	7.24 (6.93–7.47)	7.14 (6.93–7.35)	7.34 (7.16–7.47)	0.003
Serum bicarbonate (mM)	12.9 (4.0–28.0)	6.8 (4.0–9.8)	17.8 (5.0–28.0)	<0.001

[a]For comparison between patients with and without renal injury; mean values compared by Student's unpaired t test.

ance values of 17–70 ml/min are reported. After the initiation of fomepizole treatment, urinary oxalate excretion markedly decreased due to the reduced overall EG metabolism, irrespective of whether signs of renal injury were present.

Estimates of systemic exposure from case reports

Because of the relatively rapid metabolism of EG patients may present with low levels of EG but with significant plasma concentrations of GA, and such a presentation is more likely at longer periods between EG ingestion and blood analysis (except in the case of coingestion of ethanol which would inhibit the metabolism of EG). The data analysis of EG poisoning cases is generally limited due to the unknown amounts ingested, failure to analyze EG early (prior to GA formation), insufficient information on the kinetics of endogenous elimination, and the effects of treatment measures.

Table 4 summarizes a number of case reports that contain measurements of systemic exposure considered to be analytically reliable. Emphasis is on initial presentation rather than on the clinical monitoring under treatment. There is no consistent ratio between EG and GA concentrations as early as admission due to the variable periods between EG ingestion and serum analysis. As a consequence measurements of EG alone would inadequately reflect the severity of initial intoxication and are not suited to establish a no-effect level or define a potentially toxic level of exposure. Patients reported to have presented with high levels of EG (up to 55.3 mM) and GA below detection limit exemplify this statement. These patients had ethanol present in serum at the time of admission, explaining both the absence of GA and of significant symptoms (Porter et al. 1999). On the other hand, if therapeutic strategy is based on the EG concentration, appropriate measures (ethanol or fomepizole) are generally recommended at plasma EG levels higher than 3.2 mM (>20 mg/dl; Porter et al. 1999). This value is considered the threshold of toxicity for systemic exposure, including renal failure.

Reliable information is lacking in most cases of EG intoxication regarding the amounts ingested, and due to the complex toxicokinetics and lack of an appropriate physiologically based pharmacokinetic model the analytical data obtained on admission are insufficient for back-calculation of dose levels. Notwithstanding these difficulties, the data presented in Table 4 suggest that exposures much higher than the estimated threshold of 3.2 mM favorably respond to therapeutic intervention.

Conclusion

The available data on human exposures to EG refer largely to acute poisoning cases, and therefore their value for the assessment of dose-response is limited. Despite this circumstance the literature base is consistent

Table 4 Initial ethylene glycol (*EG*) and glycolic acid (*GA*) serum values derived from case reports (*n.s.* not specified)

No. of patients	Age (years)	Sex	Time from ingestion to admission (h)	EG (mM)	GA (mM)	Analytical methods EG GA	Remarks	Reference
2	2	F	1.5	22.9	12.2	GC HPLC	Recovered on ethanol infusion; multiple organ failure after 4 weeks in spite of ethanol infusion and hemodialysis	Hewlett et al. 1986
	46	M	6–8	3.1	15.4			
2	36	F	>6	40.9	21.3	GC HPLC	Partial recovery (residual renal insufficiency) upon ethanol infusion and hemodialysis	Jacobsen et al. 1988
	38	F	ca. 10	56.4	22.4			
2	37	M	4	110	<0.5	GLC GLC-MS	Recovered on ethanol i.v. and hemodialysis; acute renal failure, but recovered on hemodialysis	Malmlund et al. 1991
	22	M	24	7	29			
10	Mean 49 (range 28–73)	n.s.	Mean 11.5 (range 3.5–21.5)	Mean 18.5 (range 0.8–62.2)	Mean 17.0 (range 10.0–23.7)	n.s. GC	Fomepizole i.v. (15 mg/kg initial dose) followed by hemodialysis in 9 patients	Moreau et al. 1998
19	Mean 41 (range 19–73)	2 M, 17 F	Mean 11.4 (range 6.6–20.8)	Mean 19.8 (range 3.9–71.8)	Mean 11.8 (range 0–34.9)	GC GC	Fomepizole generally maintained at 15–30 μg/ml; hemodialysis conducted in 17/19 cases; renal injury developed in 9 patients with plasma glycolate ≥12.9 mM	Brent et al. 1999
5	n.s.	n.s.	n.s. up to 24	6.6–43.9	13.8–38.0	GC-MS	Five fatal outcomes in spite of ethanol infusion and hemodialysis attributed to high GA conc.	Porter et al. 1999

in the estimate of the minimum lethal dose in humans at about 1600 mg/kg body weight. Tolerable levels of ingested EG are derived from animal data. They indicate a NOAEL of approx. 200 mg/kg body weight/day for nephrotoxicity as critical endpoint. Aircraft de-icing workers systemically exposed to an estimated 27 mg/kg EG did not demonstrate any kidney (or other) effects.

GA, the only toxic metabolite that occurs to any extent in human plasma, mainly determines the toxicity of EG. Case reports suggest that a plasma GA concentration of about 10 mM (76 mg/dl) does not cause nephrotoxicity in humans. On the other hand, plasma EG levels of about 3.2 mM (20 mg/dl) are considered a threshold of toxicity for systemic exposure to EG. Because the severity of EG toxicity is more closely correlated with the plasma concentration of GA than with that of EG, the simultaneous determination of both parameters is important for diagnosis and therapeutic management. Analytical methods appropriate for this purpose are reviewed. Although animal data are used in comparative toxicokinetics, they cannot be extrapolated directly to predict human response. Studies in vitro demonstrated human liver to be more effective at further metabolizing GA than liver from rats or rabbits, suggesting that humans would accumulate less GA than laboratory animals following exposure to similar amounts of EG.

There appears to be sufficient evidence from human and animal data that tolerable levels of EG exposure exist. However, this consideration should not detract from initiating appropriate preventive or therapeutic measures.

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